# Protein crosslinking by 1,8-naphthalimides: influence of the 4substituent

#### R. Jeremy Woods, Jianxing Zhang, Charalene R. Green, and Robert R. Kane\*

Department of Chemistry and Biochemistry and the Center for Drug Discovery, Baylor University, P.O. Box 97348, Waco, TX 76798-7348 E-mail: Bob Kane@baylor.edu

# Dedicated to Professor Henry J. Shine on his 80<sup>th</sup> birthday

(received 07 Sept 03; accepted 27 Oct 03; published on the web 21 Nov 03)

#### Abstract

4-Alkylamino-1,8-naphthalimides have been previously demonstrated to photochemically crosslink proteins and bond tissues. In order to gain a greater understanding of protein crosslinking by these agents, and in hopes of developing improved protein crosslinking and tissue bonding agents, we have studied the effect of changing the C4 substituent on the naphthalimide chromaphore. In that regard, five compounds (compounds **5-9**; Figure 2) have been synthesized and characterized as protein crosslinking agents. Substituents that stabilize a positive charge by resonance were found to reduce protein crosslinking. However, these substituents also move the absorbance of the compounds further into the visible, which is desirable for the clinical application of these compounds to tissue binding. Further work is underway to better delineate the characteristics of an optimized photochemical tissue bonding agent.

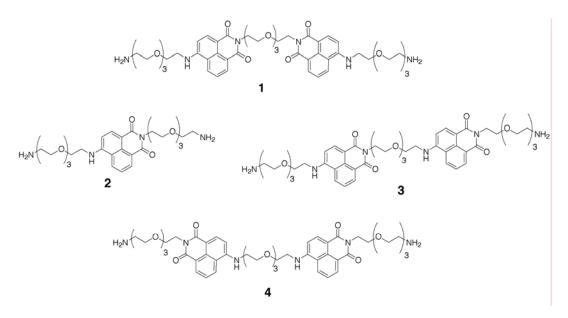
Keywords: Photochemistry, protein crosslinking, naphthalimide

### Introduction

Protein crosslinking mediated by photosensitizers is often a result of photo-oxidation of reactive amino acid residues by either singlet oxygen, the triplet state sensitizer, or other radical species formed during photolysis.<sup>1,2</sup> Subsequent dark reactions of the oxidized amino acid residues with amino acids (oxidized or unmodified) on another protein results in the formation of intermolecular crosslinks. While protein crosslinking using photosensitizers has potential utility in a variety of applications, our laboratory is especially interested in the observation that mechanical tissue bonding can be accomplished by the use of photooxidants and light.

In one study that employed this methodology, flavin mononucleotide (FMN), which has been shown to oxidatively dimerize tyrosine by a type 1 mechanism,<sup>3</sup> was demonstrated to photochemically effect the non-thermal bonding of rabbit uteral tissue ex vivo.<sup>4</sup> The bond formed using that sensitizer was more robust than could be obtained using sutures, and was approximately equal in strength to the bond that could be obtained by heating the tissue with a laser ("thermal laser tissue welding"). Similarly, rose bengal, a known photochemical source of singlet oxygen, has been shown to afford significant mechanical tissue bonding upon photolysis in a porcine skin graft model.<sup>5</sup> In research directly related to the studies described in this manuscript, a series of papers have described in vitro and in vivo experiments that establish the usefulness of the *bis*-4-alkylamino-1,8-naphthalimide derivative 1 (Figure 1) in tissue bonding applications.<sup>6-9</sup> In one such study, artificial lesions were created in knee menisci of Barbados sheep<sup>6,8</sup> and subsequently photochemically repaired using a mixture of naphthalimides related to compound **1**.<sup>10</sup> These animals returned to normal activity shortly after the surgery, although the untreated animals exhibited a substantial limp. Follow-up studies showed continued bonding and a significant healing response in the damaged area of the photochemically treated specimens, while untreated control lesions remained non-bonded and unhealed.<sup>6</sup>

Besides the tissue bonding experiments, *in vitro* experiments demonstrated that the mixture of naphthalimides used in the *in vivo* experimentation was able to photochemically crosslink suspensions of insoluble type I collagen from bovine Achilles tendon.<sup>9</sup> Later studies in our laboratory demonstrated the abilities of compound **1** and its three monomeric and dimeric derivatives 2 - 4 (Figure 1) to photochemically crosslink the model protein ribonuclease A (RNase).<sup>11</sup> The results from those studies revealed that the monomeric derivative **2** was a more efficient protein crosslinking agent than the dimeric compounds **1**, **3**, and **4**.



**Figure 1.** Structures of compounds 1 - 4.

We have recently explored the protein crosslinking activities of naphthalimides with various substituents at the 4-position. We herein describe the syntheses of five 1,8-naphthalimide derivatives, including the unsubstituted parent compound **5** (Figure 2) as well as compounds with bromo (**6**), ethoxy (**7**), ethylsulfanyl (**8**), and ethylamino (**9**) substituents at C-4. This manuscript also reports investigations into the relative abilities of these compounds to effect the photochemical crosslinking of the model protein RNase A.

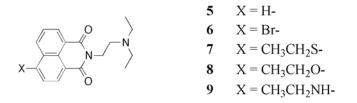
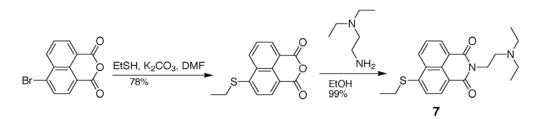


Figure 2. Structures of compounds 5-9.

### **Results and Discussion**

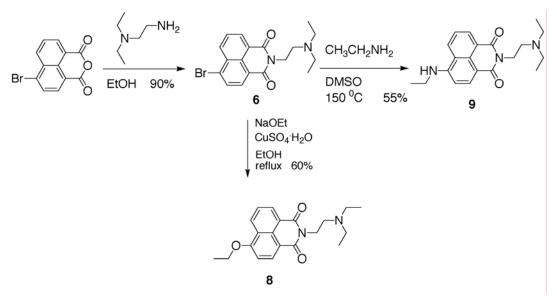
#### **Syntheses**

Naphthalimides 6 - 9 were readily synthesized using 4-bromo-1,8-naphthalic anhydride as the common starting material. As shown in Scheme 1, 7 was synthesized by a reaction of the anhydride with ethanethiol followed by condensation with N,N-diethylethylenediamine.



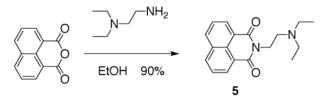
#### Scheme 1. Synthesis of compound 7.

The syntheses of **8** and **9** began with the condensation of 4-bromo-1,8-naphthalic anhydride with *N*,*N*-diethylethylenediamine to form bromo naphthalimide **6** (Scheme 2). Reaction of **6** with the sodium ethoxide and copper sulfate<sup>12</sup> afforded compound **8** in good yield, while condensation of **6** with ethyl amine provided compound **9**.



Scheme 2. Syntheses of compounds 8 and 9.

Naphthalimide **5**, which has previously been reported,<sup>13</sup> was prepared in one step by a reaction of 1,8-naphthalic anhydride with N,N-diethylethylenediamine, as shown in Scheme 3.



Scheme 3. Synthesis of compound 5.

Each compound synthesized was completely characterized by NMR and high resolution mass spectrometry, and reverse phase HPLC was used to verify the purity of each sample. Additionally, the absorption maxima for the long wavelength absorption of each compound, and the corresponding extinction coefficients, were determined. This data is shown in Table 1.

Table 1.  $\lambda_{max}$  values and extinction coefficients for the 1,8-naphthalimide derivatives

Naphthalimide	$\lambda_{max}$ (nm)	Extinction coefficient $(mM^{-1}cm^{-1})$
5	344	14.69
6	348	16.83
7	403	10.43
8	378	14.65
9	453	14.45

#### **Protein crosslinking studies**

Naphthalimides **5-9** were evaluated for their relative abilities to photochemically crosslink the protein RNase A in solution.<sup>14</sup> Solutions containing each naphthalimide and RNase A were prepared and irradiated with a Xenon arc lamp for various durations. The resulting mixtures were analyzed by polyacrylamide gel electrophoresis, and the crosslinked protein fractions were quantitated according to the intensity of the Coomassie stained bands. Plots of time vs. percent crosslinking were prepared for each naphthalimide (for an example see Figure 3), and slopes were calculated for the linear region of each plot.

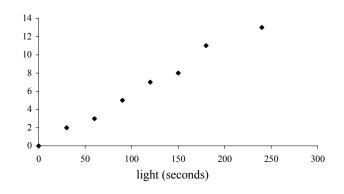


Figure 3. Crosslinking of RNase by the 4-alkylamino-1,8-naphthalimide 9 as a function of time.

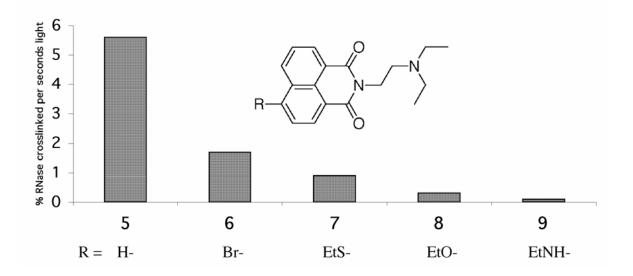
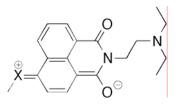
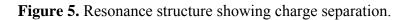


Figure 4. Initial RNase crosslinking rates for various representative 1,8-naphthalimides.

Of the five compounds examined in this study, the non-substituted naphthalimide **5** is clearly most efficient for the photochemical crosslinking of RNase, while the ethylamino-substituted naphthalimide **9** is least effective. The observed trend in protein crosslinking does not correlate with substituent electronegativity or the overall electronic character (electron rich/poor) of the

naphthalene chromaphore. However, it is known that compounds like **9** have significant charge separation in the first excited singlet state,<sup>15</sup> which is readily appreciated by examining resonance structures of the ground state like that shown in Figure 5. Compound **5** is obviously unable to participate in this type of resonance-based electron donation. In fact, the observed trend for protein crosslinking correlates well with resonance parameters for the stabilization of





electron deficient centers derived from Hammett substituent constants.<sup>16</sup> For example, as the R<sup>+</sup> values for the naphthalimide substituents become more negative, and charge separation becomes more favored, the rate of protein crosslinking decreases (Table 2). This suggests that putting resonance donors on carbon 4 decreases the propensity for these compounds to crosslink proteins. Although this is undoubtedly a gross oversimplification of the complex photochemistry that occurs with these compounds, it may provide an empirical design strategy for the construction of more potent protein crosslinkers, and perhaps, improved tissue bonding agents.

Unfortunately, for this technology to become a valid approach to tissue repair, it must operate in the visible wavelength regime, in order to avoid concomitant tissue damage and loss of

	Compound (Substituent <sup>a</sup> )					
	<b>5</b> (H-)	<b>6</b> (Br-)	7 (RS-)	<b>8</b> (RO-)	<b>9</b> (RNH-)	
Relative initial						
rate of RNase	56	17	9	3	1	
crosslinking						
Resonance						
Parameter R <sup>+</sup>	0.00	-0.30	-0.83	-1.07	-1.78	
(from ref 16)						

**Table 2.** Resonance parameter R<sup>+</sup> values and protein crosslinking propensities

<sup>a</sup> The alkyl substituents used in deriving the resonance parameter were *methyl* groups, not *ethyl*. Other parameter sets show that this change would have a minimal impact on the absolute numbers, and none at all on the trend.<sup>16</sup>

irradiation intensity due to absorption and refraction of the light. While the 4-alkylaminosubstituted chromaphore absorbs in the visible ( $\lambda_{max}$ =453 nm), the other compounds, which have much poorer resonance contributing substituents, absorb light at shorter wavelengths. For example, the unsubstituted compound **5** (which was the best protein crosslinker) absorbs well into the UV ( $\lambda_{max}$ =342 nm).

In conclusion, we have synthesized and evaluated five variously-substituted naphthalimides as protein crosslinking agents. While substituents that can stabilize a positive charge by resonance retard the protein crosslinking reaction, these types of substituents also move the absorbance of the compounds further into the visible range, a desirable characteristic for the potential clinical uses of this technology for tissue bonding. Further work is underway to better delineate the characteristics of an optimized photochemical tissue bonding agent.

### **Experimental Section**

**General Procedures.** Methylene chloride and methanol were obtained from commercial sources and were distilled prior to use. Ribonuclease A was obtained from Sigma Biochemicals. Polyacrylamide gels (Gradipore brand 4-20% Tris-glycine iGels) were obtained from VWR. **Instruments.** An EXFO Ultracure 100ss 100W xenon short arc lamp system with the light output filtered to 320-500 nm was used for irradiation. Light output from the lamp was routinely measured with an EXFO handheld radiometer. A Bio-Rad mini-protean 3 cell electrophoresis system with a VWR 105 power supply was used for electrophoresis. Reverse phase HPLC was performed on a Beckman System Gold LC. The column was a 33 mm x 7 mm Alltech Altima 'Rocket' C18 column; solvent A was 0.1% aqueous trifluoroacetic acid (TFA), solvent B was 0.1% TFA in 9:1 CH<sub>3</sub>CN:water. The flow rate was 2.5 mL/min and the linear gradient 5-90% B over 7 minutes; detection was at 254 nm.

**2-[2-(Diethylamino)ethyl]-1***H***-benz[de]isoquinoline-1,3(2***H***)-dione (5).<sup>10</sup> To a 10 mL suspension of 1,8-naphthalic anhydride (200 mg, 1.01 mmol) in ethanol was added 1.1 equivalents** *N***,***N***-diethylethylenediamine (0.1560 mL, 1.110 mmol) while the suspension was being stirred. The suspension was stirred at room temperature in the dark for one hour until the solid fully dissolved. The ethanol was then removed under reduced pressure. Flash column chromatography (silica gel, 94:6 MeCl<sub>2</sub>/ MeOH; Rf = 0.2) yielded the pure product as a light yellow solid (119 mg, 40% yield): <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) \delta 1.10 (t, 6H,** *J* **= 7.1 Hz), 2.68 (q, 4H,** *J* **= 7.2 Hz), 2.79 (m, 2H), 4.28 (m, 2H), 7.72 (t, 2H,** *J* **= 7.4 Hz), 8.18 (dd, 2H,** *J* **= 8.4, 1.0 Hz), 8.55 (dd, 2H,** *J* **= 7.2, 0.9 Hz); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>) \delta 12.20, 37.84, 47.64, 49.71, 122.55, 126.91, 128.05, 131.14, 131.51, 133.91, 164.11; DCI/NH<sub>3</sub>-HRMS** *m/z* **297.1589 (MH<sup>+</sup>) (C<sub>18</sub>H<sub>21</sub>N<sub>2</sub>O<sub>2</sub>S requires 297.1603, \Delta = -4.6 ppm); \lambda\_{max} = 342 nm; HPLC retention time 3.25 min. <b>6-Bromo-2-[2-(diethylamino)ethyl]-1***H***-benz[de]isoquinoline-1,3(2***H***)-dione (6). To a 50 mL suspension of 4-bromo-1,8-naphthalic anhydride (1.00 g, 3.61 mmol) in ethanol was added 1.1 equivalents** *N***,***N***-diethylethylenediamine (0.558 mL, 3.97 mmol) while the suspension was being stirred. The suspension was stirred under argon at room temperature in the dark for 1 hour while** 

a clear brown solution formed. The ethanol was removed under reduced pressure after verification by TLC that all of the 4-bromo-1,8-naphthalic anhydride was consumed. Flash column chromatography (silica gel, 9.5:0.5 MeCl<sub>2</sub>/MeOH) yielded the pure product as a light brown solid (1.06 g, 78% yield): <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  1.09 (t, 6H, *J* = 7.2 Hz), 2.67 (q, 4H, *J* = 7.2 Hz), 2.79 (m, 2H), 4.29 (m, 2H), 7.85 (dd, 1H, *J* = 8.5, 7.3 Hz), 8.03 (d, 1H, *J* = 7.9 Hz), 8.41 (d, 1H, *J* = 7.9 Hz), 8.57 (dd, 1H, *J* = 8.5, 1.1 Hz), 8.65 (dd, 1H, *J* = 7.3. 1.1 Hz); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>)  $\delta$  12.18, 38.14, 47.60, 49.79, 122.24, 123.11, 128.10, 129.03, 130.28, 130.66, 131.12, 131.21, 132.02, 133.29, 163.62; DEI-HRMS *m/z* 374.0628 (M) (C<sub>18</sub>H<sub>19</sub>N<sub>2</sub>O<sub>2</sub>Br requires 374.0630,  $\Delta$  = -0.5 ppm);  $\lambda_{max}$  = 348 nm; HPLC retention time 4.15 min.

**4-Ethylsulfanyl-1,8-naphthalic anhydride.** Anhydrous potassium carbonate (1.9 equivalents, 0.945 g, 6.84 mmol) and 4 equivalents of ethanethiol (1.066 mL, 0.895 g, 14.4 mmol) were added to a 50 mL solution of 4-bromo-1,8-naphthalic anhydride (1.0 g, 3.6 mmol) in anhydrous DMF. The solution was placed under argon and stirred at room temperature in the dark for 19 hours. The solution was then poured onto 300 mL ice water to give a yellow suspension. This suspension was stirred for 2 hours and the yellow solid was recovered by vacuum filtration. Recrystallization from ethanol afforded the pure product as a yellow solid (0.72 g, 78% yield): mp 197–198 °C; <sup>1</sup>H NMR (300 MHz, acetone-*d*<sub>6</sub>)  $\delta$  1.48 (t, 3H, *J* = 7.3 Hz), 3.36 (q, 2H, *J* = 7.4 Hz), 7.82 (d, 1H, *J* = 8.0 Hz), 7.95 (dd, 1H, *J* = 8.5, 7.3 Hz), 8.45 (d, 1H, *J* = 8.0 Hz), 8.61 (dd, 1H, *J* = 7.3, 1.1 Hz), 8.66 (dd, 1H, *J* = 8.5, 1.1 Hz); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>)  $\delta$  13.36, 26.19, 114.47, 119.29, 122.24, 127.00, 129.46, 130.50 131.45, 132.95, 133.71, 148.09, 160.60, 160.79; DEI-HRMS *m*/z 258.0349 (M) (C<sub>14</sub>H<sub>10</sub>O<sub>3</sub>S requires 258.0351,  $\Delta$  = -0.6 ppm).

**2-[2-(Diethylamino)ethyl]-6-ethylsulfanyl-1***H***-benz[de]isoquinoline-1,3(2***H***)-dione (7).** To a suspension of 4-ethylsulfanyl-1,8-naphthalic anhydride (0.72 g, 2.79 mmol) in 17 mL ethanol was added two equivalents of *N*,*N*-diethylethylenediamine (0.79 mL, 0.69 g, 5.58 mmol). The mixture was stirred at room temperature under argon in the dark for 3 hours, during which time all of the solid dissolved. Removal of the solvent and excess amine under vacuum gave the pure product (0.99 g, 99% yield): mp 98–99 °C; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  1.10 (t, 6H, *J* = 7.1 Hz), 1.49 (t, 3H, *J* = 7.4 Hz), 2.68 (q, 4H, *J* = 7.1 Hz), 2.78 (t, 2H, *J* = 7.6 Hz), 3.20 (q, 2H, *J* = 7.4 Hz), 4.28 (t, 2H, *J* = 7.6 Hz), 7.53 (d, 1H, *J* = 7.9 Hz), 7.74 (dd, 1H, *J* = 8.4, 7.3 Hz), 8.47 (d, 1H, *J* = 7.9 Hz), 8.56 (dd, 1H, *J* = 8.4, 1.1 Hz), 8.62 (dd, 1H, *J* = 7.3, 1.1 Hz); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>)  $\delta$  12.65, 13.95, 26.73, 38.33, 48.04, 50.21, 119.41, 122.97, 123.49, 126.98, 128.76, 129.92, 130.50, 131.17, 131.89, 145.71, 164.47, 164.49; FAB-HRMS *m/z* 357.1638 (M<sup>+</sup>) (C<sub>20</sub>H<sub>25</sub>N<sub>2</sub>O<sub>2</sub>S requires 357.1637,  $\Delta$  = 0.1 ppm);  $\lambda_{max}$  = 403 nm; HPLC retention time 4.50 min.

**2-[2-(Diethylamino)ethyl]-6-ethoxy-1H-benz[de]isoquinoline-1,3(2H)-dione (8).** A solution 6 (300 mg, 0.799 mmol) was prepared in 8 mL ethanol, followed by the addition of eight equivalents of sodium ethoxide (6.39 mmol, 435.0 mg, 2.386 mL of a 21% (wt) solution in ethanol) and 0.125 equivalents  $CuSO_4 \cdot 5H_2O$  (0.100 mmol, 24.9 mg). The solution was refluxed and stirred for eight hours. This was followed by reaction at room temperature for an additional 10 hours. The solution was then diluted with 50 mL  $CH_2Cl_2$ , washed three times with 1M NaOH, washed once with saturated NaCl, dried over MgSO<sub>4</sub>, and filtered. Removal of the solvents

under reduced pressure yielded the crude product, which was purified by silica gel flash column chromatography (97:3 CH<sub>2</sub>Cl<sub>2</sub>/CH<sub>3</sub>OH) to give the pure product as a yellow oil (164 mg, 60% yield): <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  1.12 (t, 6H, *J* = 7.1 Hz), 1.61 (t, 3H, *J* = 7.0 Hz), 2.69 (q, 4H, *J* = 7.2 Hz), 2.79 (m, 2H), 4.27 (m, 2H), 4.33 (q, 2H, *J* = 7.0 Hz), 6.99 (d, 1H, *J* = 8.3 Hz), 7.67 (dd, 1H, *J* = 8.3, 7.4 Hz), 8.49 (d, 1H, *J* = 8.3 Hz), 8.55 (dd, 1H, *J* = 8.5, 1.1 Hz), 8.56 (dd, 1H, *J* = 7.2, 1.1 Hz); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>)  $\delta$  12.60, 14.99, 38.06, 48.07, 50.17, 65.09, 106.15, 115.08, 122.66, 123.87, 126.17, 129.15, 129.75, 131.84, 133.85, 160.61, 164.29, 164.90; DEI-HRMS *m*/*z* 340.1786 (M) (C<sub>20</sub>H<sub>24</sub>N<sub>2</sub>O<sub>3</sub> requires 340.1787,  $\Delta$  = -0.2 ppm);  $\lambda_{max}$  = 378 nm; HPLC retention time 4.28 min.

**2-[2-(Diethylamino)ethyl]-6-ethylamino-1***H*-benz[de]isoquinoline-1,3(2*H*)-dione (9). Compound **6** (0.5230 g, 1.39 mmol), was combined with 15 mL DMSO and 2 equivalents ethylamine (2.78 mmol, 179.07 mg of a 70% solution in water) in a sealed tube. The solution was heated to 150 °C in an oil bath and stirred for 8 hours. This was followed by reaction at room temperature for an additional 13 hours. The solvents were then removed under vacuum and the crude product was purified by flash chromatography (silica gel, 9:1 MeCl<sub>2</sub>/MeOH) to give an orange solid (36.2 mg, 50% yield from **6**). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  1.17 (t, 6H, *J* = 7.1 Hz), 1.47 (t, 3H, *J* = 7.2), 2.73 (q, 4H, *J* = 7.2 Hz), 2.85 (m, 2H), 3.47 (qd, 2H, *J* = 7.1, 5.1 Hz), 4.31 (m, 2H), 5.34 (bs, 1H), 6.71 (d, 1H, *J* = 8.5 Hz), 7.61 (dd, 1H, *J* = 8.4, 7.4 Hz), 8.13 (dd, 1H, *J* = 8.5, 1.0 Hz), 8.45 (d, 1H, *J* = 8.4 Hz), 8.57 (dd, 1H, *J* = 7.3, 1.0 Hz); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>)  $\delta$  11.94, 14.28, 37.22, 38.32, 40.99, 47.68, 49.62, 104.32, 120.16, 123.03, 124.62, 125.94, 129.81, 131.05, 134.46, 149.44, 164.01, 164.61; DEI-HRMS *m/z* 339.1945 (M) (C<sub>20</sub>H<sub>25</sub>N<sub>3</sub>O<sub>2</sub> requires 339.1947,  $\Delta$  = -0.4 ppm);  $\lambda_{max}$  = 453 nm; HPLC retention time 3.73 min.

**Photochemical crosslinking of RNase.** PBS buffered solutions of 30  $\mu$ l each were prepared to contain 0.235 mM RNase and 0.05 mM naphthalimide. Microcentrifuge tubes (1.5 mL) were used as reaction vessels. Solutions were irradiated at 550 mW (2.82 W/cm<sup>2</sup>), with the light guide positioned at 5 mm above the solution. To help minimize heating, the tubes were inserted into a room-temperature water bath during irradiation. All samples were analyzed by SDS-polyacrylamide gel electrophoresis.

**Polyacrylamide gel electrophoresis.** Samples were diluted with an equivalent volume of 2x SDS-tris-HCl-(2-mercaptoethanol) sample buffer. The solutions were heated at 100 °C for 5 minutes in a boiling water bath, cooled on ice, and loaded onto a 4-20% polyacrylamide gel. Loading volumes for each sample were 5  $\mu$ l. The running buffer was refrigerated before use, and electrophoresis was carried out at 155 V for 1 hour and 55 minutes. After electrophoresis, the gels were stained with Coomassie blue for 12 hours and destained with a solution of 85:5:10 water/glycerol/acetic acid. Densitometry measurements of digital gel images were made with the Macintosh version of the public domain NIH Image software (developed at the U.S. National Institutes of Health and available on the Internet at <a href="http://rsb.info.nih.gov/nih-image/">http://rsb.info.nih.gov/nih-image/</a>). The mass composition of each band was calculated through the use of a RNase standard that was present on each gel.

# Acknowledgments

We wish to thank the Welch Foundation (Grant AA-1355) for financial support. RRK wishes to thank Professor Shine for serving as an inspiring model of the ideal teacher/scholar, and for realizing that sometimes people are not as dim as they might act.

# **References and Notes**

- 1. Jori, G. Photochem. Photobiol. 1975, 21, 463.
- 2. Verweij, H.; Dubbelman, T.M.A.R.; van Steveninck, J. Biochem. Biophys. Acta 1981, 647, 87.
- 3. Shen, H.-R.; Spikes, J.D.; Smith, C.J.; Kopeček, J. J. Photochem. Photobiol. A: Chem. 2000, 133, 115.
- 4. Merguerian, P.A.; Pugach, J.L.; Lilge, L.D. SPIE Proc. 1999, 3590, 194.
- 5. Chan, B.P.; Kochevar, I.E.; Redmond, R.W. Journal of Surgical Research 2002, 108, 77.
- 6. Judy, M.M.; Jackson, R.W.; Nosir, H.R.; Matthews, J.L.; Loyd, J.D.; Lewis, D.E.; Utecht, R.E.; Yuan, D. SPIE Proc. **1997**, 2970, 257.
- 7. Judy, M.M.; Nosir, H.; Jackson, R.W.; Matthews, J.L.; Lewis, D.E.; Utecht, R.E.; Yuan, D. *SPIE Proc.* **1996**, *2671*, 251.
- Judy, M.M.; Jackson, R.W.; Nosir, H.R.; Matthews, J.L.; Lewis, D.E.; Utecht, R.E.; Yuan, D. SPIE Proc. 1996, 2922, 436.
- 9. Judy, M.M.; Chen, L.; Fuh, L.; Nosir, H.; Jackson, R.W.; Matthews, J.L.; Lewis, D.E.; Utecht, R.E.; Yuan, D. SPIE Proc. **1996**, *2681*, 53.
- 10. At the time those studies were performed it was believed that the bonding agent was pure compound **1**. More recent analyses have demonstrated that the bonding agent was composed of a mixture of compounds formed in the solvolytic condensation of the diamine and bromoanhydride starting materials.
- 11. Zhang, J.; Woods, R.J.; Brown, P.B.; Lee, K.D.; Kane, R.R. Bioorg. Med. Chem. Lett. 2002, 12, 853.
- 12. Adam, W.; Qian, X.; Saha-Möller, C.R. Tetrahedron 1993, 49, 417.
- 13. Miller, K.E.; Grace, J.M.; MacDonald, T.L. Bioorg. Med. Chem. Lett. 1994, 4, 1643.
- 14. The protein crosslinking trends reported herein for RNase also hold for other proteins (data not shown).
- 15. de Silva, A.P.; Gunaratne, H.Q.N.; Habib-Jiwan, J.L.; McCoy, C.P.; Rice, T.E.; Soumillion, J.P. Angew. Chem. Int. Ed. Engl. 1995, 34, 1728.
- 16. Hansch, C.; Leo, A.; Taft, R.W. Chem. Rev. 1991, 91(2), 165.